Intestinal Colonization with Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* in Different Populations in Switzerland: Prevalence, Risk Factors and Molecular Features

João Pires[1,2], Esther Kuenzli[3,4], Christoph Hauser[5], Regula Tinguely[1], Sara Kasraian[1], Andrew Atkinson[5], Andri Rauch[5], Hansjakob Furrer[5], Vincent Perreten[6], Jonas Marschall[5], Christoph Hatz[3,4] and Andrea Endimiani[1*]

[1]Institute for Infectious Diseases (IFIK), University of Bern, [2]Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, [3]Swiss Tropical and Public Health Institute, Basel, [4]Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, [5]Department of Infectious Diseases, Bern University Hospital and University of Bern, [6]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Running title: Gut Colonization with cephalosporin-resistant *Enterobacteriaceae* in Switzerland

Key words: colonization, people, intestinal, ESBL, CTX-M-15, healthcare workers, HIV

***Corresponding author:**

Prof. Andrea Endimiani MD, PhD

Institute for Infectious Diseases, University of Bern

Friedbühlstrasse 51, CH-3010, Bern, Switzerland

Phone: +41-31-632-8632; Fax: +41-31-632-8766

Emails: andrea.endimiani@ifik.unibe.ch; aendimiani@gmail.com

Sir,

The worldwide increase of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R-Ent) colonizing the gut of healthy humans is alarming as this is a risk factor to develop future extra-intestinal infections. Colonization has been linked to previous antibiotic consumption and travel to high prevalence areas, but little is known about other populations including those with work-related exposure or with particular predisposing conditions [1, 2]. Therefore, in this study we aimed to estimate the prevalence and identify risk factors for intestinal colonization with ESC-R-Ent in volunteers from different populations in Switzerland.

Between July 2013 and November 2016, 337 volunteers living in Switzerland were enrolled in the study. These belonged to the following groups: A, HIV-positive (HIV+) individuals included in the Swiss HIV Cohort (http://www.shcs.ch/) (n=101); B, personnel of the human clinical laboratory of the Institute for Infectious Diseases, University of Bern (n=18); C, personnel from veterinary clinics and clinical or research laboratories from the Vetsuisse Faculty, University of Bern (n=164); D, health-care workers from the Department of Infectious Diseases, Bern University Hospital (n=14); and E, healthy volunteers not belonging to any of the previous groups (n=40). Volunteers filled in an epidemiological questionnaire.

Stools were enriched overnight in Luria-Bertani broth in different conditions to detect ESC-R-Ent [3]. At least five colonies were tested per each positive growth on selective plates. Species identification was obtained by using the MALDI-TOF MS (Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility tests (i.e., MICs) were obtained using the microdilution Sentitre™ GNX2F plate (TREK Diagnostic Systems, Independence, Ohio, USA) and interpreted with the 2016 EUCAST breakpoints (v6.0; www.eucast.org). β-lactamase genes (*bla*) were identified using the CT103XL microarray (Check-Points, Wageningen, The Netherlands) with subsequent PCR and sequencing. Bacterial genotypes were established with MLST (http://mlst.ucc.ie/mlst/dbs/Ecoli) and phylogenetic group (PhG) determination for *E. coli* isolates [3]. Statistical analysis was performed comparing colonized and non-colonized individuals' lifestyle factors using GraphPad

Prism version 7.0 (La Jolla, California, USA). Continuous variables were analyzed using Mann–Whitney *U* test, whereas categorical variables with Fisher's exact test. P values bellow 0.05 were considered statistically significant. Odds Ratios (ORs) and Confidence Intervals (CIs) 95% were computed for categorical variables.

The overall prevalence of ESC-R-Ent colonization in our study was 7.1% (CI 95% 4.8%-10.4%, n=24/337) and was as follows for each of the different groups: A, 6.9% (CI 95% 3.4%-13.6%; n=7/101); B, 5.6% (CI 95% 0.3%-25.8%; n=1/18); C, 7.3% (CI 95% 4.2%-12.4%; n=12/164); D, 0% (CI 95% 0%-21.5%; n=0/14); and E, 10% (CI 95% 4%-23.1%; n=4/40). Our results are consistent with those identified in surrounding countries [1]. However, the data suggest that the prevalence of ESC-R-Ent is steadily increasing as previous Swiss studies reported colonization rates between 2.8%-5.8% [1, 4].

A total of 28 ESC-R-Ent were recovered, of which 89.3% (n=25) were *E. coli*, and the remaining were *K. pneumoniae*, *E. cloacae* and *E. fergusonii* (each, n=1) (Table 1). Three individuals were found to be colonized with more than one ESC-R-Ent: two subjects possessed two different *E. coli* and another one had two *E. coli* and one *E. fergusonii*. Overall, for the ESC-R-Ent resistance to non-β-lactams was high given that 53.6% were resistant to doxycycline, 46.4% to trimethoprim-sulphamethoxazole, 39.3% to aminoglycosides, and 32.1% to fluoroquinolones. Apart from for aminoglycosides, resistance to non-β-lactams was lower than previously reported in Switzerland [4].

As shown in Table 1, ESBLs were the most frequent resistance mechanism identified (96.4%), with CTX-M-15 being the most common (67.9%). *E. coli* population structure revealed that most isolates belong to PhG A (36%), but also to B2 or D (28% each), and to lesser extent B1 (8%). Interestingly, MLST identified High Risk Clones (HiRC, 46.4%), including B2-ST131, B2-ST73, B2-ST127, D-ST648, D-ST405, D-ST69, A-ST10 and A-ST410. The identification of HiRC in the gut is concerning since these bacteria frequently cause infections, posing a threat for the carriers' health [5].

A total of 202 (59.9%) questionnaires were returned. All participants from groups A, D and E provided a questionnaire, whereas from groups B and C we received one and 46, respectively. Among all studied factors, only hospitalization abroad in the past five years was significantly associated with ESC-R-Ent colonization (OR 15.5, CI 95% 2.2-101.8, P=0.02), whereas travel abroad, antibiotic intake, diet type, nor pets were associated with colonization (Supplementary Table 1). Moreover, no particular group was associated with an increased risk of being colonized with ESC-R-Ent (P=0.78). This is surprising as we would expect that people working in human or veterinary hospital/laboratory environments would be more likely to be exposed to and colonized with ESC-R-Ent. However, our findings are partially in line with a previous meta-analysis, which demonstrated that neither lifetime hospitalization nor hospitalization in the past year or contact with pets were predictors of colonization [1]. The identification of “hospitalization abroad” as a risk factor for colonization is most likely due to the high prevalence of ESC-R-Ent in foreign hospitals compared to Switzerland.

Although the studied population is not representative of the entire country, our results suggest that the prevalence of ESC-R-Ent colonizing the gut of healthy people is increasing in Switzerland, a trend also observed in surrounding countries. Moreover, hospitalization abroad seems to contribute significantly to this phenomenon. Additionally, the high proportion of HiRCs found in the gut of healthy people should raise awareness for the role of this niche in the dissemination of life-threatening pathogens.

FUNDING

This work was supported in part by the Swiss National Science Foundation (SNF; grant number 153377 to AE). João Pires is a PhD student (2014-2017) supported by the SNF.

COMPETING INTERESTS

None declared

ETHICAL APPROVAL

HIV positive individuals: Kantonale Ethikkommission Bern: Schweizerische HIV Kohortenstudie [No. 21/88]; personnel from the IFIK and Department of Infectious Diseases: evaluated as part of a risk assessment by the hospital's infection prevention program; personnel from the Vetsuisse Faculty: signed a written consent; remaining healthy individuals: Ethikkommission Nordwest- und Zentralschweiz (EKNZ 239/12).

AUTHOR CONTRIBUTION

Conception and design (AE); volunteer recruitment (EK, CH, CS, AR, HF, VP, JM, CH); acquisition of data (JP, EK, CH, CS, RT, SK); analysis of data (JP, AA, AE); drafting the work (JP, AE); critical revision of the work (JP, EK, CH, CS, AR, HF, VP, JM, AE); final approval of the manuscript (all authors).

AKNOWLEDGEMENTS

We thank Dr Cornelia Staehelin, Melanie Lacalamita and Daniela Hirter for collecting epidemiological and clinical data.

REFERENCES

[1] Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization With Extended-spectrum Beta-lactamase–Producing *Enterobacteriaceae* and Risk Factors Among Healthy Individuals: A Systematic Review and Metaanalysis. Clinical Infectious Diseases. 2016;63:310-8.

[2] Woerther P-L, Burdet C, Chachaty E, Andremont A. Trends in Human Fecal Carriage of Extended-Spectrum β-Lactamases in the Community: Toward the Globalization of CTX-M. Clinical Microbiology Reviews. 2013;26:744-58.

[3] Pires J, Kuenzli E, Kasraian S, Tinguely R, Furrer H, Hilty M, et al. Polyclonal Intestinal Colonization with Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* upon Traveling to India. Frontiers in Microbiology. 2016;7.

[4] Geser N, Stephan R, Korczak BM, Beutin L, Hächler H. Molecular Identification of Extended-Spectrum-β-Lactamase Genes from *Enterobacteriaceae* Isolated from Healthy Human Carriers in Switzerland. Antimicrobial Agents and Chemotherapy. 2012;56:1609-12.

[5] Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiology Reviews. 2011;35:736-55.

Table 1. Molecular and phenotypic features of recovered ESC-R-Ent within the different groups

Group	ID volunteer	Main β-lactamase [a]	Species	PhG-ST [b]	Antimicrobial susceptibility test results (MICs, µg/mL) [c]
A	32094	CTX-M-15	*E. coli*	$B2_3$-ST127	FOT (16), TAZ (2), FEP (8), AZT (4), GEN (>16), TOB (8)
		CTX-M-15	*E. coli*	D_2-ST405 (CC405)	TIM (64), FOT (>64), TAZ (16), FEP (16), AZT (>32), TOB (>16), AMI (≤4), CIP (>4), LEV (>16), SXT (>8/152), DOX (16)
	32117	CTX-M-15	*E. coli*	A_1-ST410 (CC23)	TIM (32), FOT (>64), TAZ (16), FEP (8), AZT (>32), CIP (>4), LEV (>16), SXT (>8/152), DOX (16)
	32315	CTX-M-15	*E. coli*	$B2_3$-ST131 (CC131)	TIM (32), FOT (32), TAZ (8), FEP (4), AZT (16), TOB (>16), CIP (>4), LEV (8)
	31643	CTX-M-14	*E. coli*	$B2_3$-ST73 (CC73)	TIM (128), FOT (>32), TAZ (4), FEP (>32), AZT (32)
	32300	CTX-M-15	*E. coli*	B1-ST5173	FOT (32), TAZ (4), FEP (4), AZT (8)
	31570	CTX-M-15	*E. coli*	A_0-ST189 (CC165)	TIM (128), FOT (32), TAZ (4), AZT (16), DOX (8)
	32388	CTX-M-8	*E. coli*	$B2_3$-1170	TIM (32), FOT (8), TAZ (2), FEP (4)
B	KM1	CTX-M-1	*E. coli*	D_2-ST38 (CC38)	TAZ (≥32), FEP (4), DOX (4)
C	2292581	CTX-M-14-like	*E. coli*	D_1-ST31 (CC31)	FOT(8), DOX (8)
	2330192	CTX-M-1-like	*E. coli*	A_1-ST1312	FOT (16), FEP (4), AZT (4), DOX (16),
	2349998	CTX-M--1-like	*E. coli*	A_1-ST1312	FOT (16), FEP (4), AZT (8), DOX (16)
	2354728	CMY-2-like	*E. coli*	$B2_3$-ST131 (CC131)	TIM (64), FOT (16), TAZ (≥32), AZT (8), DOX (8), MIN (4)
	2378367	CTX-M-15-like	*E. coli*	$B2_3$-ST131 (CC131)	TIM (32), FOT (≥64), TAZ (8), FEP (4), AZT (≥32), DOX (4), MIN (4)
		CTX-M-15-like	*E. coli*	A_0-STNEW	TIM (16), FOT (≥64), TAZ (8), FEP (4), AZT (≥32)
	2382871	CTX-M-15-like	*E. coli*	D_2-ST405 (CC405)	TIM (32), TAZ (16), FOT (≥64), AZT (≥32), GEN (≥16), TOB (4), CIP (≥4), DOX (≥32), MIN (≥32)
	2474813	CTX-M-15	*K. pneumoniae*	-	TIM (64), FOT (32), TAZ (16), FEP (4), AZT (≥32), TOB (4), CIP (≥4), LEV (4), SXT (≥8/152), DOX (16), MIN (4)
	2498698	CTX-M-1-like	*E. coli*	A_1-ST10 (CC10)	FOT (8), AZT (8), GEN (≥16), TOB (4), SXT (≥8/152), DOX (≥32), MIN (8)
		CTX-M-1-like	*E. coli*	B_1-ST641 (CC86)	TIM (32), FOT (16), TAZ (4), FEP (8), AZT (8), GEN (≥16), TOB (2), SXT (≥8/152), DOX (≥32), MIN (≥32)
		CTX-M-1-like	*E. fergusonii*	-	FOT (≥64), FEP (4), AZT (8), GEN (≥16), TOB (4), STX (≥8/152)
	2501192	CTX-M-15-like	*E. coli*	B_1-ST205 (CC205)	TIM (32), FOT (32), TAZ (8), FEP (8), AZT (≥32), MER (4), CIP (≥4), LEV (≥16), SXT (≥8/152), DOX (16)
	2502424	CTX-M-14-like	*E. coli*	A_1-ST10 (CC10)	TIM (64), FOT (8)
	2503490	SHV-12-like	*E. cloacae*	-	TIM (32), TAZ (4), FOT (≥64), AZT (≥32), GEN (≥16), TOB (≥16), AMI (8), GEN (≥16), TOB (≥16), SXT (≥8/152), DOX (≥32), MIN (≥32)
	2506378	CTX-M-14-like	*E. coli*	D_1-ST69 (CC69)	FOT (8), DOX (≥32), MIN (16),
E	29	CTX-M-14	*E. coli*	D_2-ST648 (CC648)	TIM (32), FOT (≥64), AZT (4), TOB (8), CIP (≥4), LEV (≥16), SXT (≥8/152), DOX (≥32), MIN (8)
	50	CTX-M-15	*E. coli*	$B2_3$-ST131 (CC131)	TIM (32), FOT (32), TAZ (8), FEP (4), AZT (16), GEN (≥16), TOB (≥16), LEV (8), CIP (≥4), SXT (≥8/152), DOX (4)
	59	CTX-M-15	*E. coli*	A_1-ST59	FOT (32), TAZ (4), AZT (8), CIP (2), LEV (4), SXT (≥8/152),
	HV1	CTX-M-15	*E. coli*	A_1-ST1312	FOT (8), AZT (4), SXT (≥8/152)

Notes. A, HIV+ individuals; B, personnel from human clinical laboratories; C, personnel from veterinary clinical or research laboratories; and E, others. TIM, ticarcillin/clavulanic acid; FOT, cefotaxime; TAZ, ceftazidime; FEP, cefepime; AZT, aztreonam; MERO, meropenem; AMI, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; LEV, levofloxacin; SXT, Trimethoprim/sulfamethoxazole; DOX, doxycycline, MIN, minocycline; ST, sequence type; CC, clonal complex.

[a] Only β-lactamases conferring resistance to extended-spectrum cephalosporins are shown

[b] Phylogenetic group and ST are only shown for *E. coli*

[c] MIC values were interpreted according to the EUCAST criteria (Version 6.0; www.eucast.org), except for doxycycline and minocycline for which CLSI guidelines (document M100-S26) were used. Values presented represent only those from intermediate or resistant categories